

## RESEARCH ARTICLE

# Plant growth regulators as thinners in guava orchards increase larger fruits with higher nutritional value

Marcelo de Souza Silva<sup>1</sup>, Sarita Leonel<sup>2\*</sup>, Magali Leonel<sup>3</sup>, Jackson Mirellys Azevedo Souza<sup>4</sup>,  
Rafael Bibiano Ferreira<sup>2</sup>, Rafaelly Calsavara Martins<sup>2</sup>

<sup>1</sup>Faculty of Higher Education and Integral Training, FAEF, Garça, SP, Brazil, <sup>2</sup>Department of Horticulture, School of Agriculture, Sao Paulo State University (UNESP), Botucatu, São Paulo, Brazil, <sup>3</sup>Tropical Root and Starches Center, Sao Paulo State University (UNESP), Botucatu, São Paulo, Brazil, <sup>4</sup>Department of Agronomy, Viçosa Federal University, UFV, Viçosa, MG, Brazil

## ABSTRACT

The production of large guavas guarantees better market prices. Chemical thinning is a practice performed to increase fruit size and nutritional value with benefits for growers and consumers. By using ethephon and benzyladenine at 0, 150, 300 and 450 mg L<sup>-1</sup>, fruit thinning in red guava was evaluated. The percentage of fruit setting and increase in fruit mass, marketable yield, soluble solids (SS), titratable acidity (TA), SS/TA ratio, pH, sugars, ascorbic acid, antioxidant activity, total phenolic compounds, flavonoids and pigments were evaluated. The plant growth regulators improved the setting of larger fruits. Fruit thinning higher efficiency was observed in the ethephon and benzyladenine concentrations from 300 mg L<sup>-1</sup> and upwards, however, benzyladenine induced phytotoxicity at the same concentrations. The chemical thinning can be used to promote larger fruits with better uniformity favoring higher marketability and as a source of interest compounds enhancing nutritional guava fruits value depending on the thinner and the concentration.

**Keywords:** *Psidium guajava* L.; Cytokinin; Ethylene; Bioactive compounds; Thinners.

## INTRODUCTION

Guava crop has been widely spread in tropical and subtropical regions of the world and has gained visibility in the agro-food sector due to the attractive characteristics, such as appearance, shape, aroma and nutritional value. Guava fruits have high levels of vitamin C, fibres, proteins, sugars and minerals, satisfactory levels of vitamins A and B (Adrees et al., 2010) and are also sources of carotenoids, phenolics, ellagic acid and flavonoids (Corrêa et al., 2011; Joseph and Pryia, 2011; Lima et al., 2019).

Furthermore, guava has great versatility in the food industry and fresh food market. The commercialization of fresh fruits has increased due to changes in consumers habits with the search for healthy food, which has boosted the guava domestic and export markets. In Brazil, the guava cv. Paluma is widely cultivated, showing high productivity, with sized medium fruits of red pulp, pleasant aroma, being very appreciated by consumers (Ribeiro et al., 2020; Vitti et al., 2020).

The fruit drop is one of the problems in the guava orchards. Despite an initial fruiting rate of up to 54%, some plants only have 6% of their fruit reach full maturity (Corrêa et al., 2002). In commercial guava orchards, fruit drop may mean reduced income or economic loss to the producer, and the fruit set index can be used as an indicator of production (Silva et al., 2016).

Guava growers need to invest in technologies that increase yield and fruit quality. Fruit thinning is essential for production of fruits with an adequate commercial size (Khan et al., 2014). Hand thinning has been practiced for the production of larger fruits with better prices but increases production costs, as it requires specialized labor (Pavanello and Ayubi, 2014).

The growers are interested in maximizing the potential of chemical thinners. However, the variability of results obtained may be related to a number of factors which needs to take into consideration prior to the use of thinners. Some researchers reported positive effects with the chemical

### \*Corresponding author:

Sarita Leonel, Department of Horticulture, School of Agriculture, Sao Paulo State University (UNESP), Botucatu, São Paulo, Brazil.  
E-mail: sarita.leonel@unesp.br.

Received: 02 August 2021; Accepted: 10 January 2022

thinning of the fruits using plant growth regulators (PGRs) such as ethephon (Cruz et al., 2011; Giavanaz et al., 2016; Torres et al., 2021) and benzyladenine (BA) (Ouma and Matta, 2002; Schröder et al., 2013) and in guava, with naphthalene acetic acid (Abbas et al., 2014).

Chemical thinning is beneficial for fruit growth because the relationship between the source of the collector and the collector size is regulated during the cell division period. The PGRs can positively or negatively influence some aspects of fruit growth (Link, 2000). The PGRs action mode in chemical thinning of fruits has been investigated. Exogenously applied ethephon stimulates ethylene production and triggers ethylene-dependent reactions, such as the abscission of flowers or fruits (Torres et al., 2021). According to Schröder et al. (2013) in apple the BA action mode as a thinning agent is via correlative inhibition of the polar indoleacetic acid (IAA) transport of lateral fruits caused by the increase in the polar IAA transport of the bourse shoot tips. These studies evidenced the effects of chemical thinning on yield and quality improvements.

Chemical thinning with PGRs is therefore less likely to over thin when small errors are made in concentration. The fixation of remaining fruits is the better parameter to evaluate the efficiency of chemical thinning because the relationship between smaller and larger fruit loads according to thinning can be better established (Koen and Jones, 1985; Dennis Jr., 2000). However, there is still little information about this technology for safe use in guava crop.

This research was aimed to evaluate the efficacy of ethephon and BA on guava fruit setting and its effect on marketable yield and fruit quality.

## MATERIALS AND METHODS

### Site description

The replicated trial in two crop cycles (2015/2016 and 2016/2017) was carried out at the São Manuel Experimental Farm of the São Paulo State University (UNESP), Brazil (22°44'28" S and 48°34'37" W and 740 masl). The region has a *Cfa* climate (humid subtropical climate, mesothermic). The soil was classified as a sandy-textured dystroferic Red Latosol (EMBRAPA, 2006), and the soil chemical proprieties were determined.

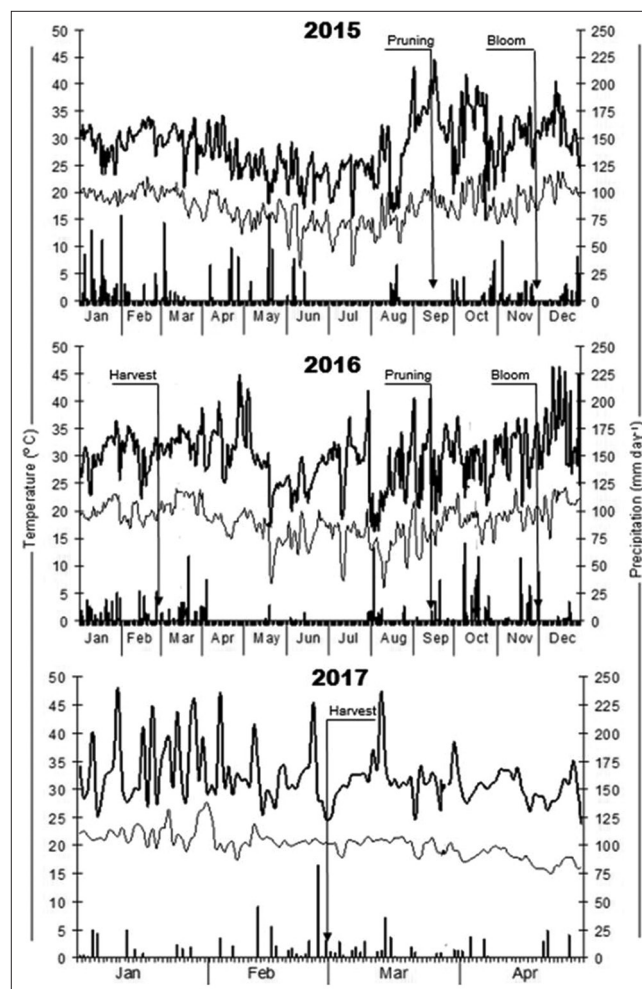
The guava crop cv. Paluma is 6 year old. Plant field spacing was 6 m between rows and 4 m between plants i.e. a stand of 416 plants ha<sup>-1</sup>. Crop management was based on the recommendations for the culture.

A weather station located within 100 m of the plots recorded air temperature, humidity, precipitation, wind speed and solar radiation (Fig. 1) ([www.fca.unesp.br](http://www.fca.unesp.br)).

### Chemical thinning

The 24% ethephon ([2-chloroethyl] phosphoric acid) (Ethephon®) and 2% benzyladenine (Na 6-benzyladenine) (Maxcel®) were applied at 0, 150, 300 and 450 mg L<sup>-1</sup> i.e., using four replicates and five plants per experimental plot. The experimental design was a randomized block in 2x4 factorial schemes, being the first factor the PGRs and the second factor the concentrations.

The thinners spraying were carried out when the guava fruits had an average size of 18 mm. Each tree received 2.5 L of spray volume, which contained different concentrations of PGRs and 3% of surfactant (Agral®). The PGRs were applied 84 and 92 days after the pruning production (Silva et al., 2016) and 56-68 days after the full bloom in the two crop cycles, respectively.



**Fig 1.** Rainfall (I), maximum (-) and minimum (-) air temperatures recorded in experimental site.

### Fruit setting, fruit mass increase percentage and marketable yield

Prior to application of the treatments, 10 productive and well distributed branches of each guava plant were identified, and the initial number of fruits was counted. Such operation was repeated 35 days after PGRs spraying, when there was stabilization of thinned fruits number and fixed fruits number. The percentage of fruit remaining in guava plant was calculated by Equation 1.

$$\% \text{ setting } = 100 - \left[ \frac{n_0 - n_{35}}{n_0} \right] * 100 \quad (1)$$

Where  $n_0$  = initial number of fruits (number of fruit),  $n_{35}$  = number of fruits 35 days after application of the treatments (number of fruit).

The fruits were harvested from the stage 4 of maturation (yellowish-greenish peel color), as recommended by Cavalini et al. (2015). Based on commercial classification of guava for the market (CEAGESP, 2020), fruits with equatorial diameter  $\leq 50$  mm were removed in the two crop cycles. Marketable yield was obtained by the mass of classified fruits and the planting density. The percentage of increase in the mass of the fruit was calculated in relation to the mass of control fruit.

### Fruit quality

In the evaluation of the fruit quality, 20 fruits were selected randomly by repetition, totaling 80 fruits per treatment. The whole fruits (peel and pulp) were crushed in a mixer (Philips Walita Viva Collection - RI1364) to obtain an homogeneous extract.

### pH

The potential for hydrogen (pH) was measured in homogenized fruit extracts (50 g) by using the digital pH meter (DMPH-2). Titratable acidity (TA) was performed by titration with 0.1 N sodium hydroxide (NaOH) in a solution of 1 g of the homogenized fruit extract, 50 mL of distilled water and 0.3 mL of phenolphthalein, expressed as a percentage of citric acid.

### Soluble solids

Soluble solids (SS) were measured with a digital refractometer (Atago 3405 PR-32a, PALETTE Series) using 1 g of the homogenized fruit extract and expressed as °Brix.

### SS/TA ratio

The ratio was calculated by the relationship between the soluble solids content and the titratable acidity (Ribeiro et al., 2020).

### Sugars

The sugar content in the fruit pulp was estimated by the Lane and Eynon methodology (Anjum et al., 2020).

### Reducing sugars

The fruit extract was mixed with 100 mL of distilled water, 10 mL of 20% potassium oxalate and 25 mL of 25% solution of lead acetate. The solution was designated as filtrate. Drops of methylene blue (1%) were added to the filtrate and titration with Fehling's solution (10 mL) was carried out under constant boiling until the appearance of a red color. Percentage of reducing sugars was calculated by Equation 2.

$$\text{Reducing sugars (\%)} = 6.25 * \left( \frac{A}{B} \right) \quad (2)$$

Where A = volume of standard sucrose titrated (mL), B = volume of the aliquot of samples titrated (mL).

### Total sugars

25 mL of filtrate were added 20 mL of distilled water and 99.9% HCl (5 mL) with overnight incubation to complete hydrolysis. The solution was neutralized with 0.1 N NaOH and titrated with Fehling's solution (10 mL). Percentage of total sugars was calculated by Equation 3.

$$\text{Total sugars (\%)} = 25 * \left( \frac{A}{B} \right) \quad (3)$$

### Non-reducing sugars

Non-reducing sugars were calculated by Equation 4.

$$\text{Non reducing sugars (\%)} = 0.95 * (\text{total sugars} - \text{reducing sugars}) \quad (4)$$

### Ascorbic acid

The ascorbic acid contents were determined according to the Association of Official Analytic Chemists method 967.21 (AOAC, 2019). The homogenized extract of guava (10 g) was diluted in 100 mL of 3% metaphosphoric acid and passing through the filter paper. Next, 5 mL of filtrate was taken and titrated with 2,6-dichlorophenol iodophenol (DCPIP) as an indicator. The results were expressed as mg 100 g<sup>-1</sup>. The standardization curve was made by titration and was performed with the standard solutions containing a known amount of ascorbic acid.

### Antioxidant activity

The antioxidant activity (% reduced DPPH): 100 mg of the sample was diluted in 3 mL of acetone, taken to a shaker

for 40 min. After that, the samples were centrifuged for 15 min at 4 °C and 15,000 rpm, from which 150 µL were collected to react with 2850 µL of the DPPH solution for 40 min, with spectrophotometer reading at 515 nm (Brand-Williams et al., 1995).

The total phenolic content was determined using the Folin-Ciocalteu colorimetric method (Swain and Hills, 1959). Samples of 100 mg macerated and frozen in liquid nitrogen were diluted in 3 mL of pure methanol and incubated for 30 min in an ultrasonic bath. Subsequently, the samples were centrifuged for 15 min at 14,000 rpm and 4 °C and the supernatant collected for analysis. The absorbance value at 765 nm was obtained in tests on an UV-Vis SP 2000 BEL Photonics® spectrometer and compared with a calibration curve obtained for gallic acid, expressed as mg 100 g<sup>-1</sup> gallic acid equivalents (GAE) in fresh weight (FW). All steps were performed in the dark.

### Total flavonoid

The total flavonoid content (mg of routine 100 g<sup>-1</sup> sample): 300 mg of fresh sample was initially weighed and 4 mL of the 15% acidified MeOH solution was added. The samples were taken to the ultrasonic bath at 30 °C for 30 min. Thereafter, they were centrifuged for 30 min under rotation at 6000 rpm at 5 °C. The supernatants were collected and 1 mL of the aluminium chloride solution was added. After 50 min of reaction in the dark, the readings were carried out at 425 nm (Awad et al., 2000).

### Pigments

Pigments (mg 100 g<sup>-1</sup> sample) were determined in extracts with 100 mg of fresh pulp and homogenized in acetone. The samples were centrifuged for 10 min, 4 °C, 2000 rpm. The readings were performed on the spectrophotometer at a wavelength of 537 nm for total anthocyanins and 470 nm for total carotenoids. All steps were performed in a dark environment (Sims and Gamon, 2002).

### Statistical analysis

The two years of data for variables evaluated were analyzed as repeated measures. The data were submitted to analysis of variance. When significant, the data related to the factor “thinners” (Benzyladenine and Ethephon) were submitted to the Tukey test, while the data referring to the factor “concentrations of PGRs” was analysed by means of regression ( $p < 0.01$  and  $p < 0.05$ ). The data for fruit setting percentage and fruit mass increase percentage were transformed to  $\arcsin \sqrt{x/100}$ .

The coefficients of Pearson's linear correlation ( $p$ ) were estimated to define the degree of association between the treatments and the evaluated variables (Equation 5).

$$p = \frac{\sum_{i=1}^n (X_i - \bar{X})(Y_i - \bar{Y})}{(n-1)S_x S_y} \quad (5)$$

Where  $n$  = number of observations,  
 $X_i$  and  $Y_i$  = the sample means of  $\bar{X}$  and  $\bar{Y}$ , respectively,  
 $S_x$  and  $S_y$  = the standard deviations of  $X$  and  $Y$ , respectively.

## RESULTS AND DISCUSSION

### Fruit setting

The analysis of the percentage of fruits that remained on the plants after treatment with PGRs presented an interaction of the two factors, PGR and concentrations. Linear decreases were observed in the fixed fruits such as the increase in the concentrations of PGRs. PGRs differed in the concentration of 300 m L<sup>-1</sup>, when ethephon had less effect on fruit setting than benzyladenine (Figs. 2 and 3a).

The thinning effect of BA can be explained by promoting the restriction of photoassimilates translocation to leaves and fruits and reducing the polar transport of auxin in the fruit stalk region, which results in greater sensitivity of the abscission layer to effect of endogenous ethylene (Zwack et al., 2013). The other hypothesis is that the BA thinners enhance cell division leading to larger fruit at harvest and are considered to be mild thinners, can be used jointly with other products to obtain desired thinning effect (Mahmood et al., 2016).

Dennis Jr (2000) reported that the BA application stimulates the production of ethylene in leaves and fruits and the setting rate increases with the applied concentration. However, despite the positive effect of BA on fruit setting, with the application of 300 mg L<sup>-1</sup> and 450 mg L<sup>-1</sup>, tanning symptoms were observed in leaves and fruits (Fig. 4). This is a problem for the commercialization for natural consumption, as it makes them less attractive and depreciates their price. Kretzschmar et al. (2007), also observed these symptoms in apples trees, indicating

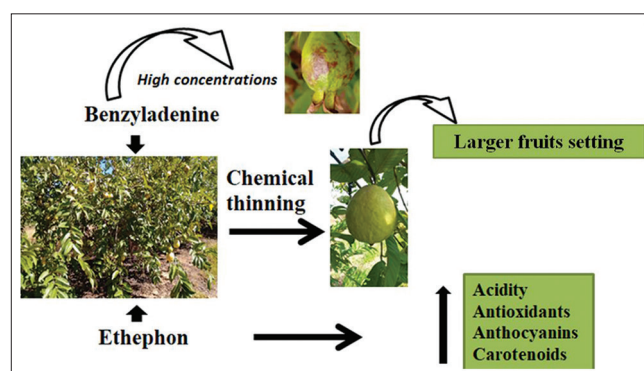
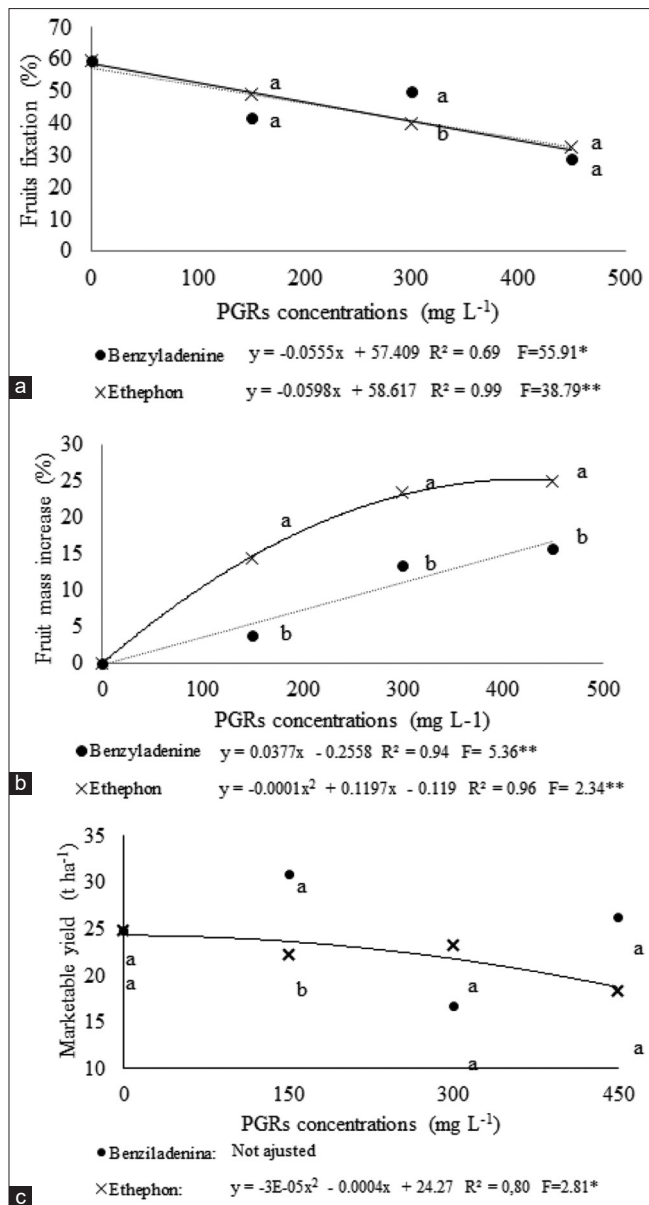


Fig 2. General diagram of the experiment.

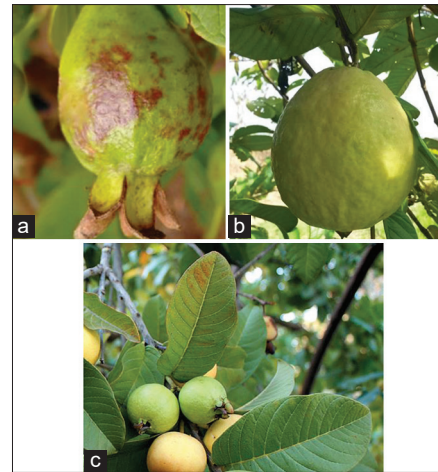




**Fig 3.** Fruit setting percentage (a); Percentage of increase fruit mass (b); Marketable yield (c). Same letters among PGRs did not differ by the Tukey test ( $p < 0.05$ ).

the need for adjustments in BA concentrations, maybe decreasing the concentrations intervals. Note that the smaller the concentration to be applied the lower the costs with the thinner acquisition, representing a saving for the guava grower.

The decrease in fruit setting in the highest concentrations of ethephon is possibly associated that high concentrations of ethylene in the vegetal tissue increases the sensitivity of the layer of abscission to ethylene, triggering the fall of the organs due to the synthesis of enzymes that degrade the cell wall, such as cellulase and polygalacturonase (Iqbal et al., 2017).



**Fig 4.** Images of fruits under PGRs spraying. a) Phytotoxicity symptom in fruits sprayed with higher concentrations of benzyladenine. b) Marketable fruit. c) Fruits with inappropriate sizes for target market.

Ethephon are absorbed by the tissues is hydrolysed to release ethylene, and this gas induce abscission of leaves in some plants. Thus, this could be the basis for its thinning effect. The role of ethylene in the thinning action of other chemicals remains controversial (Dennis Jr., 2000). The risk of over thinning is much higher with this product (Mahmood et al., 2016). Nevertheless, in this study no defoliation and over thinning were observed in all the ethephon concentrations.

The fruit setting percentage is genotype and climate dependent and because of this the growers may be reluctant to make full use of thinners because of variable results that have occurred from the use of a thinner or the fear of over thinning. This demonstrates the need for more researches and that extensive tests should be conducted to determine the optimum concentration and timings of application (Mahmood et al., 2016). Highlighting that good results were obtained with two applications of naphthalenoacetic acid at 400 mg L<sup>-1</sup> which promoted the flowers thinning of 64.9% in guava cv Gola. In the region the Faisalabad, Pakistan, the practice of chemical thinning during flowering of the plants is aimed at avoiding production in the summer, a time of increased incidence of pests and diseases (Abbas et al., 2014).

Corrêa et al., (2011) emphasized that climate has a great influence in the fruit set index in guava which is a climatic dependent factor. According to the fruit pruning time the cultivars presented the higher or smaller index. The cultivar Pedro Sato pruned in the beginning of summer presented 32.36% and Paluma and Rica cvs presented 18.7% and 12.2%, respectively, when pruned at winter. The information about this is important in crop prediction and fruit quality.

The data obtained in this experiment reinforced the influence of climate. The full bloom occurred in a period of low rainfall in the first crop cycle, which resulted in a greater fruit drop for both PGRs evaluated. On the other hand, in the second evaluation cycle, moderate precipitation, associated with average temperatures around 20-23°C, promoted an increase in the percentage of fruit setting, which resulted in a higher yield of marketable fruits (Fig. 1).

### Fruit mass and marketable yield

Both PGRs increased the fruit mass. The increase in fruit mass presented linear increases for BA from 3.83% (150 mg L<sup>-1</sup>) to 15.64% (450 mg L<sup>-1</sup>). For ethephon, the increments were quadratic: 14.31% (150 mg L<sup>-1</sup>) to 27.05% (450 mg L<sup>-1</sup>) (Fig. 3b).

The increase in fruit mass at the expense of fewer fruits is related to less competition between drains. There is an increase in the growth rate and in the final size of the remaining fruits due to the better balance between source and drain in the plant (Abbas et al., 2014; Giovanaz et al., 2016).

The ethephon (450 mg L<sup>-1</sup>) presented a marketable yield 22.93% less than obtained without application (Fig. 3c). However, with this same concentration there was an increase of 27.05% in the fruit mass. Since larger fruits are destined for more demanding markets, they are consequently sold at high prices, which justify the application of this type of technique. Nevertheless, its application should be avoided when there is no significant gain in fruit size.

The averages of marketable yield did not adjust to regression models when using BA (Fig. 3c), allowing suggesting the need for further studies evaluating lower concentrations and application times for 'Paluma' guava. The differences in marketable yield between PGRs and concentrations can be occurred as a function of greater number of fruits within the standards required by the market (CEAGESP, 2020), while in other concentrations most fruits presented inappropriate size for target market (Fig. 4). The lower marketable yield was influenced by the small size of the fruits, which probably had greater

competition for photoassimilates. Similar results were reported by Cruz et al., (2018) in 'Poncan' mandarin with spraying ethephon 600 mg L<sup>-1</sup>.

Ethephon and BA are considered post bloom thinners (Dennis Jr., 2000) and the mode of action for each one is different. The BA has the ability to thin fruit and enhance cell division. The enhance cell division can lead to larger fruit at harvest (Mahmood et al., 2016). Ethephon can thin apples when applied 10 to 20 days after bloom and remove fruitlets that are greater than 12.7 mm in diameter (Ouma and Matta, 2002). The differences in the data obtained in the present study, with the PGRs and their concentrations may be due to the time of the thinners spraying, with fruits in the appropriate size (18 mm in diameter) for ethephon. However the BA sprayings possibly could be earliest with smaller fruits, favouring cell division and the increase of larger fruits.

### Fruit quality

The analysis of the data obtained for the quality parameters of the guava fruits presented that there were no effects of the two PGRs for pH, soluble solids, ratio, total sugars, ascorbic acid and polyphenols (Table 1).

The spraying of ethephon resulted in the highest averages of titratable acidity (0.66%) and benzyladenine presented 0.62%. The acidity of the guava fruits reflects directly on their sensorial quality, whereas, they also indicate the harvest stage. According to Link (2000) sugar and acid content are improved by increasing thinning intensity. The author reported that in yours trials with apple cultivars fruits thinned gave 2-3% more soluble solids and 10-20% titratable acid contents compared to unthinned.

The interaction between PGRs and concentrations on the percentage of reducing sugars (glucose and fructose), for ethephon concentrations, presented maximum value with 223.8 mg L<sup>-1</sup> (Fig. 5a). Comparing the two PGRs for reducing sugars (%) only at 150 and 300 mg L<sup>-1</sup> was observed difference. The fruits treated with ethephon had the highest values. Ethylene is the precursor of the maturation process, which justifies the greater availability

**Table 1: F-values, degrees of freedom (DF), coefficients of variation (CV) and means of qualitative attributes of 'Paluma' guava fruits after chemical thinning**

	DF	F value					
		pH	SS	MI	TS	Ascorbic acid	Polyphenols
Block	3	0.86 <sup>ns</sup>	0.25 <sup>ns</sup>	0.76 <sup>ns</sup>	0.52 <sup>ns</sup>	0.53 <sup>ns</sup>	0.82 <sup>ns</sup>
PGRs (A)	1	3.52 <sup>ns</sup>	0.31 <sup>ns</sup>	2.67 <sup>ns</sup>	0.20 <sup>ns</sup>	2.20 <sup>ns</sup>	2.97 <sup>ns</sup>
Concentrations (B)	3	1.69 <sup>ns</sup>	0.58 <sup>ns</sup>	2.60 <sup>ns</sup>	0.41 <sup>ns</sup>	0.80 <sup>ns</sup>	0.84 <sup>ns</sup>
AxB	3	1.19 <sup>ns</sup>	1.58 <sup>ns</sup>	1.33 <sup>ns</sup>	0.91 <sup>ns</sup>	1.57 <sup>ns</sup>	1.30 <sup>ns</sup>
CV		7.15	4.28	7.15	18.96	8.42	28.16
Mean		16.82	10.69	16.82	10.19	139.28	779.83

SS = Soluble solids; MI = Maturation index and TS = Total sugars.

of soluble sugars in the fruits of the treatments with ethephon at 150 and 300 mg L<sup>-1</sup>, in relation to BA.

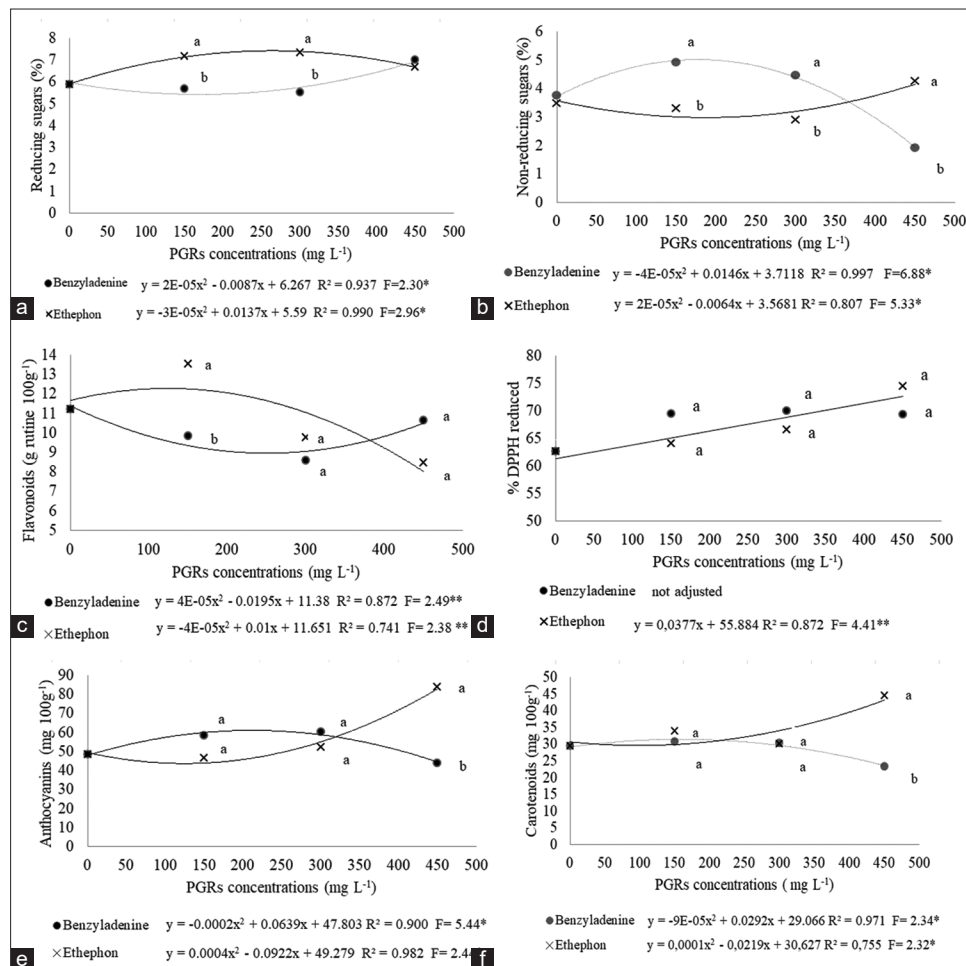
Percentage of non-reducing sugars increased with the BA concentrations, with a maximum value of 182.5 mg L<sup>-1</sup> (Fig. 5b). A similar effect was observed by Ouma and Matta (2002) in the chemical thinning of apples with benzyladenine (75 mg L<sup>-1</sup>) and carbaryl (0.2%).

The spraying of higher concentrations of PGRs led to an increase in the levels of non-reducing sugars, especially in fruits sprayed with ethephon. Ethephon induces ripening (Dennis Jr., 2000; Iqbal et al., 2017) and both PGRs are associated with the possibility of increasing the capacity of tissues as physiological drains, by increasing the demand for photoassimilates and the ratio of the source and drain partition. In addition, cytokinins regulate the activity of the extracellular invertase enzyme and a hexose transporter, and this hexose transporter is responsible for promoting the entry of hexoses into the drain cell (Iqbal et al., 2017).

PGRs had effects on flavonoid levels, with a significant increase with the use of ethephon. The spraying of BA reduced the levels of flavonoids in the fruits, with an increase in the concentration of 450 mg L<sup>-1</sup>, although no differ from the control (Fig. 5c). Guava fruit contain high amounts of phytochemicals that have the ability to defend against free radicals to prevent chronic and degenerative diseases (Suwanwonga and Boonpangrak, 2021). Del'Arco and Sylos (2017) reported that the antioxidant activity of flavonoids is even greater than that of vitamins E and C.

The antioxidant activity of guavas presented increasing linear effect with the increase of the ethephon concentrations. The positive effect of ethephon in the chemical thinning of guava fruits was evidenced by enhancing of the antioxidant activity. The ethephon 450 mg L<sup>-1</sup> resulted in a higher value of reduced DPPH (74.5%), i.e. higher antioxidant activity (Fig. 5d).

The nutritional properties of guava together with the increasing interest of this fruit are recognized indicating



**Fig 5.** Reducing sugars (a), non-reducing sugars (b), flavonoids (c), antioxidant activity (d), anthocyanins (e) and carotenoids (f) of 'Paluma' guava fruits as a function of the application of PGRs and concentrations in the chemical thinning of fruits. Same letters among PGRs did not differ by the Tukey test ( $p < 0.05$ ).

the great potential of nutraceutical use (Ho et al., 2012) and guava can be called a superfruit due to its concentrations of antioxidants (Lima et al., 2019). Therefore, there is a need for appropriate cultural management techniques, which will enable the greater expression of interest compounds, with the adequate composition of antioxidants for the fruit consumption. However, it is important to highlight that the antioxidant activity and the phytochemical composition of guava fruits can vary significantly depending on the cultivar, the colour of the fruit pulp and the pre and post-harvest treatments (Flores et al., 2015).

The data were adjusted for quadratic anthocyanins and carotenoids with the concentrations evaluated (Figs. 5e and 5f), with a maximum value of 165.6 mg L<sup>-1</sup> for anthocyanin's and linear decreasing for the carotenoid content. The means comparison between the PGRs demonstrated that the highest values for anthocyanins and carotenoids were observed in fruits submitted to chemical thinning with ethephon in relation to BA, at 450 mg L<sup>-1</sup> (Fig. 5c and 5d). Summarizing the ethephon enabled higher acidity, reducing sugars, antioxidant activity, anthocyanins and carotenoids contents in the fruits in the highest concentration (450 mg L<sup>-1</sup>).

Anthocyanins and carotenoids also have beneficial effects on health promotion due to the prevention of some diseases. This is due to action in the sequestration of reactive oxygen species, highlighting antioxidant, antiplatelet, anti-inflammatory, hypertensive and hypoglycaemic action (Freire et al., 2012). Carotenoids are also prominent because they are vitamin A precursors, and this conversion occurs naturally in the liver (Cruz et al., 2018).

### Correlation among traits

There was a significant correlation between the concentrations of benzyladenine (0.78) and for ethephon (0.87) for the fruit setting percentage (Table 2). Negative effect between the averages of marketable yield and the ethephon concentrations (-0.43) was observed. Ethephon concentrations increased the chemical thinning of the fruits and decreased marketable yield. The increase in the PGRs concentrations led to a higher percentage of fruits aborted.

The primary effects of chemical thinning on fruit quality are primarily altering crop load. Fruit mass increase percentage was directly related to thinning intensity (Link, 2000). The highest percentage of chemical thinning and fruit mass increase percentage was positively correlated with the increase in the thinners concentrations. Since carbon based compounds are the source of fruit growth, and the supply of carbon available to the fruit may be limiting during early development by competitions from

**Table 2: Pearson correlation coefficients between the PGRs concentrations of 'Paluma' guava fruits submitted to chemical thinning**

	Plant growth regulators	
	Benzyladenine	Ethephon
% Fruit setting	0.78**	0.87**
% Increase mass fruit	0.50*	0.53*
Marketable yield	-0.14 <sup>ns</sup>	-0.04*
Soluble solids (°Brix)	-0.53*	0.20 <sup>ns</sup>
Titrateable acidity (%)	0.23 <sup>ns</sup>	0.63 <sup>ns</sup>
Maturation index (SS/TA)	-0.60*	-0.16 <sup>ns</sup>
pH	-0.24 <sup>ns</sup>	0.40 <sup>ns</sup>
Reducing sugars (%)	0.31 <sup>ns</sup>	0.32 <sup>ns</sup>
Non-reducing sugars (%)	-0.34 <sup>ns</sup>	0.24 <sup>ns</sup>
Total sugars (%)	-0.24 <sup>ns</sup>	0.37 <sup>ns</sup>
Ascorbic acid (mg 100g <sup>-1</sup> )	0.20 <sup>ns</sup>	-0.32 <sup>ns</sup>
Antioxidant activity (% reduced DPPH)	-0.19 <sup>ns</sup>	0.78**
Polyphenols (mg gallic acid 100g <sup>-1</sup> )	-0.44 <sup>ns</sup>	0.13 <sup>ns</sup>
Flavonoids (g 100g <sup>-1</sup> )	-0.47 <sup>ns</sup>	-0.17 <sup>ns</sup>
Anthocyanins (mg 100g <sup>-1</sup> )	0.04 <sup>ns</sup>	0.48*
Carotenoids (mg 100g <sup>-1</sup> )	-0.64 <sup>ns</sup>	0.56*

ns = Not significant, \* and \*\* = Aignificant at 1% and 5%, respectively.

too many fruitlets, a marked influence of the thinning time on fruit mass would be expected (Link, 2000).

The quality attributes of the fruits presented effect only between the soluble solids (-0.53), maturation index (-0.60) and the BA concentrations, showing that the increase in concentrations of this PGR reduces the values of these parameters, that is, the high concentrations of BA applied are inhibiting the development of compounds bound to fruit ripening. Zwack et al., (2013) also verified a significant correlation between the exogenous application of cytokinin and the progress of the maturation process, due to the performance of this hormone in the synthesis of proteins and amino acids that delay the senescence process.

There was a negative correlation between the carotenoid content (-0.64) and the increase in BA concentrations, while there was a positive correlation between the highest antioxidant activity (0.78), anthocyanins (0.48) and carotenoids (0.56), increasing ethephon concentrations. Ethylene regulates multiple physiological, biochemical and molecular processes related to maturation, such as the biosynthesis of antioxidants and pigments compounds (Cruz et al., 2018), justifying the positive correlation by increasing these compounds with the elevation of ethephon concentrations.

All quality attributes must be evaluated for a good economic return. Establishing the trends of thinning on the quality attributes can help to select the best thinners, concentrations and spraying intervals for local conditions, typically being determined by market requirements (Link, 2000).



## CONCLUSIONS

The plant growth regulators were effectiveness on red guava larger fruits setting and the higher efficiency in thinning was observed in the ethephon and benzyladenine concentrations above 300 mg L<sup>-1</sup>. However, a phytotoxic effect of benzyladenine was observed in the highest concentrations (300 and 450 mg L<sup>-1</sup>). The remaining fruits for both PGRs showed better uniformity favouring higher marketability. The chemical thinning improved the quality profile and the functional compounds of the guava fruits depending on the plant growth regulator and the concentration. Since larger fruits are better quoted, this crop management can improve the profitability for guava growers.

## ACKNOWLEDGEMENT

This research was supported by the Brazilian National Council for Scientific and Technological Development (CNPq. Processes 304455/2017-2 and 302827/2017-0).

### Authors' contributions

All authors contributed equally to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

## REFERENCES

- Abbas, M. M., S. Ahmad and M. A. Javaid. 2014. Effect of naphthalene acetic acid on flower and fruit thinning of summer crop of guava. *J. Agric. Res.* 52: 111-116.
- Adrees, M., M. Younis, M. Farroq and K. Hussain. 2010. Nutritional quality evaluation of different guava varieties. *Pak. J. Agric. Sci.* 47: 1-4.
- AOAC. 2019. Official Methods of Analysis. Method No. 967.21. Washington, DC: Association of Official Analytical Chemists. Available from: <https://www.aoac.org/official-methods-of-analysis-21st-edition-2019>
- Anjum, M. A., H. Akram, M. Zaidi and S. Ali. 2020. Effect of gum arabic and Aloe vera gel based edible coatings in combination with plant extracts on postharvest quality and storability of "Gola" guava fruits. *Sci. Hortic.* 271: 109506.
- Awad, A. M., A. Jager and L. M. Westing. 2000. Flavonoid and chlorogenic acid levels in apple fruit: characterization of variation. *Sci. Hortic.* 83: 249-263.
- Brand-Williams, W., M. E. Cuvelier and C. Berset. 1995 Use of a free radical method to evaluate antioxidant activity. *LWT.* 28: 25-30.
- Cavalini, F. C., A. P. Jacomino, M. J. Trevisan and A. C. A. Miguel. 2015. Harvest time and quality of Kumagai and Paluma guavas. *Rev. Bras. Frutic.* 37: 64-72.
- CEAGESP. 2020. Companhia de Entrepósitos e Armazéns Gerais de São Paulo. Hortiescolha Goiaba. Available from: <http://www.ceagesp.gov.br/entrepósitos/servicos/hortiescolha/goiaba> [Last accessed on 2020 Aug 17].
- Corrêa, L. C., C. A. F. Santos, F. Vianello and G. P. P. Lima. 2011. Antioxidant content in guava (*Psidium guajava*) and araçá (*Psidium* spp.) germplasm from different Brazilian regions. *Plant Genet. Resour.* 9: 384-391.
- Corrêa, M. C. M., R. M. Prado, W. Natale, M. A. C. Silva and L. Pereira. 2002. Índice de pegamento de frutos em goiabeiras. *Rev. Bras. Frutic.* 24: 783-786.
- Cruz, M. C. M., J. D. Ramos, R. A. Moreira and V. B. Marques. 2011. Raleio químico na produção de tangerina "Ponkan". *Ver. Bras. Frutic.* 33: 279-285.
- Cruz, A. B., R. E. Bianchetti, F. R. R. Alves, E. Purgatto, L. E. P. Peres, M. Rossi and L. Freschi. 2018. Light, ethylene and auxin signaling interaction regulates carotenoid biosynthesis during tomato fruit ripening. *Front. Plant Sci.* 9: 1170-1186.
- Dennis, F. G. Jr. 2000. The history of fruit thinning. *Plant Growth Regul.* 31: 1-16.
- EMBRAPA. 2006. Sistema Brasileiro de Classificação de Solos. 2nd ed. Empresa Brasileira de Pesquisa Agropecuária, Rio de Janeiro.
- Del'Arco, A. P. W. and C. M. Sylos. 2017. Effect of industrial processing for obtaining guava paste on the antioxidant compounds of guava (*Psidium guajava* L.) 'Paluma' cv. *Rev. Bras. Frutic.* 40: e11.
- Flores, G., S. B. Wu, A. Negrin and E. J. Kenelly. 2015. Chemical composition and antioxidant activity of seven cultivars of guava (*Psidium guajava*) fruits. *Food Chem.* 170: 327-335.
- Freire, J. M., C. M. P. Abreu, A. D. Corrêa, A. A. Simão and C. M. Santos. 2012. Avaliação de compostos funcionais e atividade antioxidante em farinhas de polpa de goiabas. *Rev. Bras. Frutic.* 34: 847-852.
- Giovanaz, M. A., J. Bartz, M. S. Pasa, F. C. Chaves and J. C. Fachinello. 2016. Absciscic acid as a potential chemical thinner for peach. *Pesqui. Agropecu. Bras.* 50: 989-992.
- Ho, R., A. Violette, D. Cressend, P. Raharivelomanana, P. A. Carrupt and K. Hostettmann. 2012. Antioxidant potential and radical-scavenging effects of flavonoids from the leaves of *Psidium cattleianum* grown in French Polynesia. *Nat. Prod. Res.* 26: 274-277.
- Iqbal, N., W. A. Khan, A. Ferrante, A. Trivellini, A. Francini and M. I. R. Khan. 2017. Ethylene role in plant growth, development and senescence: Interaction with other phytohormones. *Front. Plant Sci.* 8: 1-9.
- Joseph, B. and R. Pryia. 2011. Review on nutritional, medicinal and pharmacological properties of Guava (*Psidium guajava* Linn). *Int. J. Pharm Bio Sci.* 2: 53-69.
- Khan, N., A. M. Abbasi, G. Dastagir, A. Nazir, G. M. Shah, M. M. Shah and M. H. Shah. 2014. Ethnobotanical and antimicrobial study of some selected medicinal plants used in Khyber Pakhtunkhwa (KPK) as a potential source to cure infectious diseases BMC Complement. *J. Integr. Med.* 14: 122.
- Koen, T. B. and K. M. Jones. 1985. A model of ethephon thinning of golden delicious apples. *J. Hortic. Sci.* 60: 13-19.
- Kretschmar, A. A., G. A. B. Marodin, V. Duarte, R. M. Valdebenito-Sanhueza and D. S. Guerra. 2007. Efeito de fitorreguladores sobre a incidência de podridão carpelar em maçãs "Fuji". *Ver. Bras. Frutic.* 29: 414-419.
- Lima, R. S., S. R. S. Ferreira, L. Vitali and J. M. Bloco. 2019. May the superfruit red guava and its processing waste be a potential ingredient in functional foods? *Int. Food Res. J.* 115: 451-459.
- Link, H. 2000. Significance of flower and fruit thinning on fruit quality. *Plant Growth Regul.* 31: 17-26.
- Mahmood, S., N. H. Mohamaad, S. Ali, R. A. Ripa and M. G. Hossain. 2016. Effect of plant growth regulators on fruit set and quality of guava. *Truk. Agric. Food Sci.* 4: 1088-1091.
- Ouma, G. and F. Matta. 2002. Response of several apple tree

- cultivars to chemical thinner sprays. *Afr. J. Food Sci.* 7: 16-20.
- Pavanello, A. P. and R. A. Ayub. 2014. *Raleio químico de frutos de ameixeira com ethephon*. *Ciênc. Rural*. 44: 1766-1769.
- Ribeiro, L. R., S. Leonel, J. M. A. Souza, E. L. Garcia, M. Leonel, L. N. H. Monteiro, M. S. Silva and R. B. Ferreira. 2020. Improving the nutritional value and extending shelf life of red guava by adding calcium chloride. *LWT*. 130: 109655.
- Schröder, M., H. Link and K. F. Bangerth. 2013. Correlative polar auxin transport to explain the thinning mode of action of benzyladenine on apple. *Sci. Hortic.* 153: 84-92.
- Silva, M. J. R., M. A. Tecchio, S. Domiciano, S. Leonel and R. Balestero. 2016. Phenology, yield and fruit quality of 'Paluma' guava tree at different pruning times. *Ciênc. Agrotecnol.* 40: 317-325.
- Sims, D. A. and J. A. Gamon. 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sens. Environ.* 81: 337-354.
- Suwanwonga, Y. and S. Boonpangrak. 2021. Phytochemical contents, antioxidant activity, and anticancer activity of three common guava cultivars in Thailand. *Eur. J. Integr. Med.* 42: 101290.
- Swain, T. and W. E. Hills. 1959. The phenolic constituents of *Prunus persica* domestic: The quantitative analysis of phenolic constituents. *J. Sci. Food Agric.* 10: 63-68.
- Torres, E., J. Giné-Bordonaba and L. Asín. 2021. Thinning flat peaches with ethephon and its effect on endogenous ethylene production and fruit quality. *Sci. Hortic.* 278: 109872.
- Vitti, K. A., L. M. Lima and J. G. M. Martines Filho. 2020. Agricultural and economic characterization of guava production in Brazil. *Rev. Bras. Frutic.* 42: e447.
- www.fca.unesp.br. College of Agricultural Sciences, Department of Agricultural Engineering, São Paulo State University, UNESP.
- Zwack, P. J., B. R. Robinson, M. G. Risley and A. M. Rashotte. 2013. Cytokinin response factor 6 negatively regulates leaf senescence and is induced in response to cytokinin and numerous abiotic stresses. *Plant Cell Physiol.* 54: 971-981.